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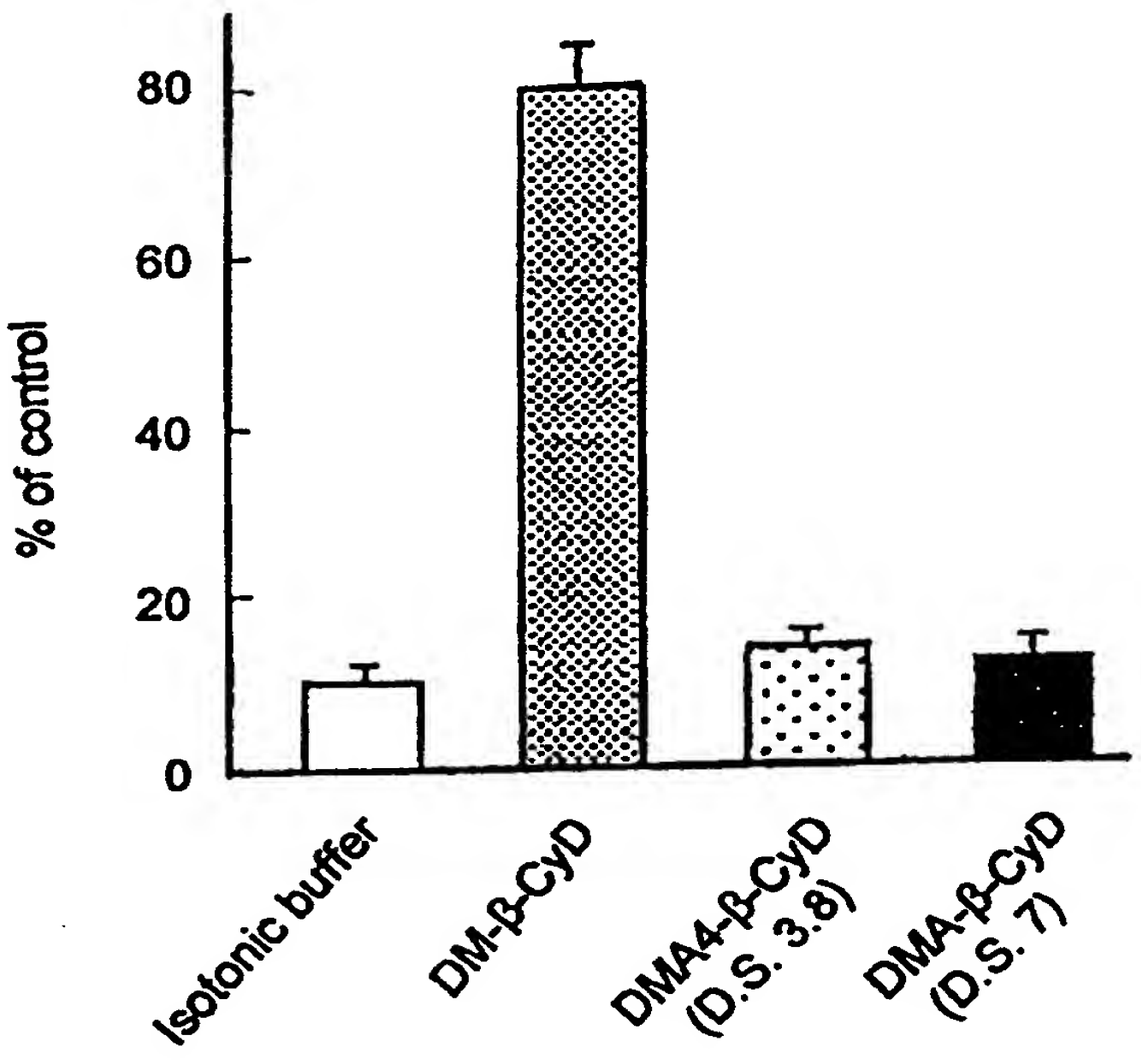
(54) Title: ACYLATED ALKYLATED CYCLODEXTRIN DERIVATIVES AND THEIR USE AS CARRIERS FOR MEDICAMENTS

(57) Abstract

Cyclodextrin derivatives having at least one lower alkyl group and at least one C_{2-20} alkanoyl group in the molecule, are disclosed pharmaceutical preparations wherein the derivative and a medicament are in such a state that they are closely compounded, are also disclosed. The cyclodextrin derivative having lowered hemolytic activity and its use as a medicament carrier.



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DESCRIPTION

ACYLATED ALKYLATED CYCLODEXTRIN DERIVATIVES AND THEIR USE AS CARRIERS FOR MEDICAMENTS

Technical Field

This invention relates to acylated alkylated cyclodextrin derivatives, and a process for preparing the same and use of the same as carriers for medicaments.

Background Art

Cyclodextrin (hereinafter also referred to as CyD) is an oligosaccharide wherein glucose residues are cyclicly bound by α-1,4 bond and composed of 6, 7 or 8 glucose residues, and ones called α, β or γ-CyD are known. Further, so-called branched cyclodextrins (hereinafter also referred to as branched CyD) are also known wherein glucosyl group(s) or maltosyl group(s) is/are α-1,6 bound to one or two of the glucose units of these CyDs.

These CyDs and branched CyDs have high inclusion ability on certain chemical substances, and are utilized for various uses such as stabilization of unstable substances, retention of volatile substances and solubilization of water-sparingly soluble or insoluble substances, in the pharmaceutical, food and cosmetic fields.

Further, in order to utilize the physicochemical characteristics and inclusion ability of the above CyDs as polyfunctional medicament carriers, etc., various CyD derivatives are provided. However, in the course of these researches, the presence of several problems in physical properties, inclusion ability, intracorporeal kinetics, economical efficiency, etc. has been pointed out, and it has come to be made clear that

there is some limitation on their use. Particularly, use thereof as medicament carriers, namely dissolution aids (solubilizers) or tissular disorder-preventing agents for injections or various preparations to be applied to the mucosae (e.g., eye drops, suppositories, etc.), on which low hemolytic activity, low actions to give topical irritation and low actions to cause tissular disorder are required, has been extremely inconvenient.

On the other hand, α-CyD has a solubility in water as comparatively high as 14.5 g/100 mL (25℃) and its hemolytic activity and muscular irritation are lower than those of β-CyD, but there is a limitation on α-CyD that the guest compounds of inclusion are limited to small molecules. Further, its price is 30 times as high as that of β-CyD and it has a disadvantage point also in an economical aspect. γ-CyD is the best among α-, β- and γ-CyD on the aspect of safety such as hemolytic activity and actions to cause tissular disorder and has inclusion ability equal to that of β-CyD, but its price is about 100 times as high as that of β-CyD and therefore it has not so been utilized from economical reason. Further, glucosylated or maltosylated branched CyDs rouse interest partially because their solubilities in water are increased compared with the corresponding unbranched CyDs, but they are not always satisfactory in behavior as carriers for the above medicaments.

Thus, attempts have been made to improve the physical properties or functionality of β-CyD, which is easy to obtain, by chemically modifying it. For example, there have been obtained thereby heptakis (2,6-di-O-methyl)-β-CyD (hereinafter referred to as DM-β-CyD) wherein the hydroxyl groups at the 2- and 6-positions of the glucose are methylated, heptakis (2,3,6-tri-O-methyl)-β-CyD (hereinafter referred to as TM-β-CyD) wherein all the hydroxyl groups at the 2-, 3- and

6-positions of the glucose are methylated, 2-hydroxypropyl-β-CyD (hereinafter referred to as HP-β-CyD) wherein a hydroxypropyl group is introduced in the hydroxyl group mainly at the 6-position of the glucose, etc. Szejtili et al. suggest that it is possible to utilize DM-β-CyD as a dissolution aid for injection (J. Incl. Phenom., 1(2), 135 (1983)).

However, this DM-β-CyD is extremely easy to dissolve in water and has strong inclusion ability, but has a problem that since its solubility and stability constant strikinly decrease at the side of high temperatures and the dissociation of the medicament from the medicament inclusion composite becomes easy, the designation of sterilization conditions for the injection is hindered. Moreover, DM-β-CyD has a stronger hemolytic activity than β-CyD, and its action to cause tissular disorder at the time of intramusclular injection is also larger than β-CyD. This tendency is the same in TM-β-CyD, and TM-β-CyD shows intermediate values between DM-β-CyD and β-CyD. On the other hand, as to HP-β-CyD, large improvement is made on the lowering of solubility and the lowering of stability constant at high temperatures, and actions to cause tissular disorder such as hemolytic activity and muscular irritation are also considerably improved compared with β-CyD, but they are equal to those of α-CyD, and it is the state of things that HP-β-CyD is far inferior to γ-CyD which has the lowest hemolytic activity and muscular irritation among natural CyDs.

Uwagama et al. disclose a pharmaceutical preparation wherein 2-hydroxyethyl-CyD (hereinafter referred to as HE-β-CyD) wherein a 2-hydroxyethyl group is introduced or 2,3-dihydroxypropyl-CyD (hereinafter referred to as DHP-β-CyD) is used as a carrier for medicaments utilizing its low hemolytic activity or action to inhibit hemolytic activity, low action to

cause tissular disorder or action to prevent tissular disorder, low action to give topical irritation or action to lower topical irritation (Japanese Laid-open Patent Publication No. 61430/1989). However, the hemolysis-inhibiting action of HE-β-CyD and DHP-β-CyD is almost equal to that of γ-CyD, and further improvement will be desired to provide them for clinical use.

Szejtili et al. propose (carboxy)alkyloxyalkyl derivatives of CyD and a pharmaceutical composition comprising such a derivative and a medicament (WO92/14762). However, there is no specific description on whether they show a sufficient hemolytic activity-inhibiting action or not.

Disclosure of Invention

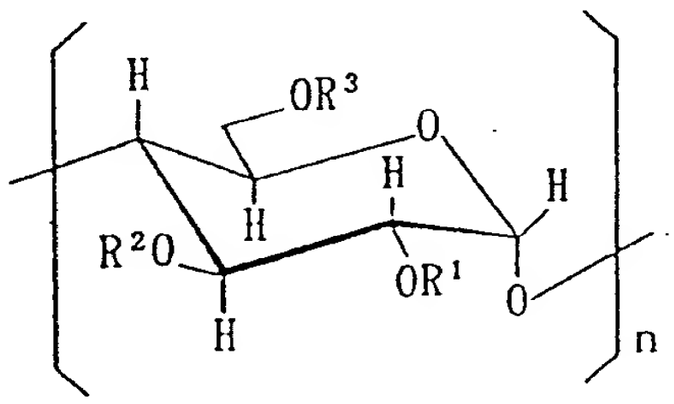
Under such a situation, it becomes very important to provide a low hemolytic medicament carrier. Namely, this is because if a low hemolytic medicament carrier, which makes it possible to administer sparingly soluble medicaments parenterally, can be provided, it can be expected, also for such a medicament that it has been thought to be impossible to apply it, to maintain its high concentration in the blood, and it is thought to make great contribution to the field of pharmacotherapy. Therefore, the objects of the invention lie in providing CyD derivatives satisfying the above needs, and providing the actual use of such a CyD derivative as a carrier or delivery tool for sparingly soluble medicaments.

For solving the above problems, the present inventors have synthesized various CyD derivatives, and examined their hemolysis-inhibiting action. As a result, they found that CyDs having an acyl group and an alkyl group together in the molecule are CyD derivatives having a hemolytic activity that is significantly lower even compared with HE-β-CyD and γ-CyD whose hemolytic

activities have hitherto been recognized to be low. It was further recognized that these derivatives sufficiently retain the medicament inclusion ability of the corresponding CyDs.

Therefore, according to the invention are provided acylated alkylated CyDs useful as solubilizers, adsorbents or agents having inclusion ability.

The acylated alkylated CyD according to the invention is, specifically, an acylated alkylated cyclodextrin derivative represented by the formula (I)



(I)

wherein n is any of integers 6, 7 and 8, and the 1-position and the 4-position of the sugar residues at the both ends are mutually bound by a covalent bond,

R^1, R^2 and R^3 independently represent hydrogen atoms, lower alkyl groups or C_{2-20} alkanoyl groups, or in some case, represent glucosyl groups or maltosyl groups whose hydroxyl group(s) may be replaced with lower alkyloxy group(s) or C_{2-20} alkanyloxy group(s),

provided that any of R^1, R^2 and R^3 of the number of total $3 \times n$ composed of each n are simultaneously at least one lower alkyl group and at least one acyl group, and the residual groups, when exist, are hydrogen atoms or the glucosyl groups or maltosyl groups of the number of up to at most 2.

There is a case where such derivatives are

provided as compounds wherein the degree of substitution of the acyl group and/or alkyl group is different or part thereof are epimerized, depending on starting materials, reaction conditions, etc. for preparing them, or there is also a case where it is convenient to provide them as a form of a mixture. Moreover, the acylated alkylated CyD derivatives sufficiently meet the objects of the invention, even in the form of mixtures, and thus such mixtures are also provided by the invention.

The acylated alkylated CyD derivatives or mixtures of two or more of the derivatives can efficiently be prepared by acylation reaction using corresponding partially alkylated CyD derivatives as starting materials. Thus, such a process for preparing an acylated alkylated CyD is also provided by the invention.

The acylated alkylated CyD derivatives or mixtures of two or more of the derivatives, even if they are derived from β-CyD, not only show hemolytic activities significantly lower compared with HE-β-CyD and γ-CyD which have been recognized to have low hemolytic activity, but sufficiently retain the inclusion ability on medicaments which parent β-CyD inherently has. Moreover, rabbit muscular irritation of the acylated alkylated CyD derivatives is much weaker than that of DM-β-CyD.

Thus, according to the invention is provided the above acylated alkylated CyD derivatives or use of the derivatives as carriers or delivery tools for water soluble, sparingly water soluble or water insoluble medicaments. As a specific embodiment of this use is provided a pharmaceutical preparation which comprises such an acylated alkylated cyclodextrin derivative or mixture of two or more of the derivatives and such a medicament in such a state that they are closely compounded. A process for preparing such a pharmaceutical

preparation is also provided.

Brief Description of Drawings

Fig. 1 is the mass spectrum (matrix: methanol, glycerol and m-nitrobenzyl alcohol, which is the same hereinafter) of DMA-β-CyD obtained in Example 1.

Fig. 2 is the ^{1}H-NMR spectrum of DMA-β-CyD obtained in Example 1.

Fig. 3 is the ^{1}H-NMR spectrum of DMA4-β-CyD obtained in Example 2.

Fig. 4 is the ^{1}H-NMR spectrum of butyrated DM-β-CyD obtained in Example 3.

Fig. 5 is the ^{1}H-NMR spectrum of octanoylated DM-β-CyD obtained in Example 4.

Fig. 6 is a drawing showing the results of the hemolytic activity test on various CyD derivatives. In figure 6, the white square (□), black triangle (▲), white triangle (△), white circle (○), black circle (●), white inverted triangle (▽), and white diamond (◇) represent DMA-β-CyD, DMA4-β-CyD, β-CyD, DM-β-CyD, TM-β-CyD, 2-HP-β-CyD with a degree of substitution (D.S.) of 4.8 and sulfobutyl ether β-CyD with a D.S. of 3.5, respectively.

Fig. 7 is a graph showing the released amounts of cholesterol from the intact erythrocytes at the time when various CyD derivatives are contacted with erythrocytes. The vertical axis represents the released amount (%) of cholesterol supposing that the amount of cholesterol in all the erythrocytes is 100 %.

Detailed Description of the Invention

The "acylated alkylated" in the invention means such a state that an acyl group and an alkyl group exist simultaneously on one molecule. Therefore, in the acylated alkylated CyD derivative in the invention, at least one of the hydroxyl groups in the CyD molecule is

converted to an acyl ester, and at least one of the other hydroxyl groups is converted to an alkyl ether.

Surprisingly, such a CyD derivative which simultaneously has an acyl group and an alkyl group on the CyD molecule has a significantly lower hemolytic activity than the corresponding CyD, as stated above. However, in view of significantly lowering hemolytic activity, in the above formula (I), is preferred a CyD derivative wherein 50 % (e.g, 7 as to β-CyD) or more of R^1 and R^3 of the number of total 2×n (e.g, 14 as to β-CyD) are lower alkyl groups, the residual R^1 and R^3 and R^2 are at least one acyl group, and the residual R^1, R^2 and R^3, when exist, are hydrogen atoms, or a mixture of two or more of the derivatives. As a further preferred one, there can be mentioned a CyD derivative wherein 50 % or more, particularly about 100% of R^1 and R^3 of the number of total 2×n are lower alkyl groups, and 50 % or more, particularly about 100% of R^2 of the number of n are C_{2-20} alkanoyl groups, or a mixture of two or more of the derivatives.

When harmony between the inclusion ability of the medicament and economical efficiency is taken into account, preferred is one which corresponds to n = 7 in the formula (I), i.e. β-CyD, and does not have a glucosyl group or maltosyl group as a branched sugar residue. Thus, as a still further preferred acylated alkylated derivative, there cas be mentioned a β-CyD derivative wherein 7 or more of 14 R^1s and R^3s are lower alkyl groups and 4 or more of 7 R^2s are C_{2-20} alkanoyl groups, or a mixture of two or more of the derivatives. As the mixture, there can, for example, be mentioned a mixture of two or more of compounds selected from the group consisting of compounds wherein 7 to 14 of all the R^1s and R^3s are alkyl groups. In this occasion, the number of R^2 which is a C_{2-20} alkanoyl group is the same or different between the two or more of compounds.

However, most preferably, there can be mentioned a β-CyD derivative wherein all of R^1s and R^3s are lower alkyl groups and all of R^2s are C_{2-20} alkanoyl groups, and a mixture wherein such derivatives are mainly (i.e, exceeding 50 %) contained.

The lower alkyl groups include straight-chain or branched alkyl groups having 1 to 6 carbon atoms, but as preferred ones, there can be mentioned methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl groups, etc., and further preferred among them is a methyl group.

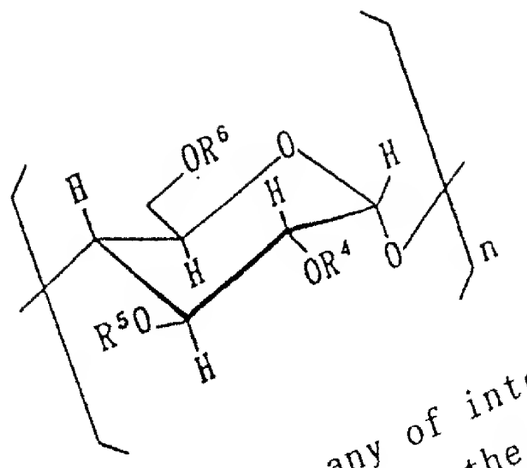
As to the C_{2-20} alkanoyl groups, the alkyl part may be straight-chain or branched, and as preferred ones, there can be mentioned acetyl, n-propanoyl, n-butanoyl, n-pentanoyl (or valeryl), n-hexanoyl (or caproyl), n-heptanoyl (or enanthoyl), n-octanoyl (or capryloyl), n-dodecanoyl (or lauroyl), n-tetradecanoyl (or myristoyl) and n-octadecanoyl (or stearoyl) groups, etc., and, above all, acetyl, n-propanoyl, n-butanoyl and n-hexanoyl are preferred, and further preferred among them is an acetyl group.

Thus, as particularly preferred ones among acylated alkylated CyD derivatives or mixtures of two or more of the derivatives according to the invention, there can be mentioned heptakis (2,6-di-O-methyl-3-acetyl)-β-CyD with a degree of substitution (D.S.) of 7 at the 3-position (hereinafter referred to as DMA-β-CyD, and the following abbreviations follow this) and mixtures mainly containing this DMA-β-CyD, or a mixture of acetylated DA-β-CyDs with a lower substitution (D.S. 3.5-6) at the 3-position.

Such an acylated alkylated CyD of the invention can be prepared by the following process, as another embodiment of the invention, which comprises reacting a partially alkylated CyD derivative represented by the formula (II)

10

(II)



wherein n is any of integers 6, 7 and 8, and the 1-position and the 4-position of the sugar residues at the both ends are mutually bound by a covalent bond,

R^4, R^5 and R^6 independently represent hydrogen atoms, lower alkyl groups, glucosyl groups or maltosyl groups, provided that R^4, R^5 and R^6 of the number of total $3 \times n$ composed of each n are, simultaneously, at least one lower alkyl group and at least one hydrogen atom, and the number of the glucosyl groups and maltosyl groups is at most 2,

or a mixture of the derivatives with an activated C_{2-20} alkanoic acid in a polar solvent, if necessary in the presence of a condensing agent.

The partially alkylated CyD derivatives of the formula (II) themselves are known or available on the market, but ones prepared according to preparation processes known per se can also be used. Further, as the activated C_{2-20} alkanoic acid, acid anhydrides or acid halides (chlorides, bromides) of alkanoic acids can be used, but preferably acid anhydrides can be mentioned. When an acid halide is used, it is desirable to make a basic organic amine such as triethylamine coexist as a hydrogen halide-capturing agent, but it is advantageous to use pyridine as a solvent and a hydrogen halide-capturing agent or condensing agent.

When an acid anhydride is used as an acylating reactant and pyridine is used as a solvent, the acyl

groups of the desired number can be introduced into a compound of the formula (II) by using pyridine in an amount enough to dissolve the CyD reactant and the acid anhydride reactant and carrying out reaction, usually, at a temperature around 80℃ for several hours to 72 hours. The desired acylated alkylated CyD derivatives can be isolated and purified from the thus obtained reaction mixture using solvent extraction, various chromatographies, and recrystallization per se known, but as stated above, they can also be separated, in a state of a mixture of two or more of the derivatives, from the reaction solvent and the unreacted reactant or the side reaction products. Usually, after the completion of the reaction, the reaction mixture is added dropwise into ice water to decompose the excess acid anhydride, and the desired CyD derivative is extracted with chloroform. Sodium carbonate is added to the extract, and the mixture is desalted and subjected to separation and purification using silica gel columns, and if necessary, subjected to recrystallization from an appropriate solvent. The desired CyD derivative can be obtained by concentrating the obtained substance to dryness. The structure of the obtained substance can be confirmed by mass spectrum, elementary analysis, etc.

As stated above, the thus obtained acylated alkylated CyD derivatives or mixtures of the derivatives of the invention have hemolytic activities and muscular irritation significantly lowered, compared with the previous CyDs, and have an action to solubilize water-sparingly soluble or insoluble medicaments at room temperature, and are useful as carriers or delivery tools for such medicaments.

Therefore, as another embodiment of the invention, there is provided a pharmaceutical preparation which comprises such an acylated alkylated CyD derivative or a mixture of two or more of the derivatives and

such a medicament in such a state that they are closely compounded. The "state that they are closely compounded" means such a state that the CyD derivative and the medicament are homogeneously mixed or such a state that the medicament and the CyD derivative form an inclusion compound.

The preparation in such a state can be prepared by sufficiently kneading the CyD derivative and the medicament, in an aqueous solvent (including a mixed solvent between methanol, ethanol, acetonitrile, dimethylformamide or the like and water), in such a state that the CyD derivative and the medicament are suspended or dissolved, using a kneader or the like regularly used for the preparation of formulations.

The pharmaceutical preparation can be administered in an administration form such as parenteral administration, namely intravenous injection, intramuscular injection, subcutaneous injection or topical administration to the skin or mucosa., but administration methods are not limited thereto, and it can also be administered by oral administration.

Medicaments or active ingredients applicable to the preparation according to the invention may be any medicaments including water-soluble or sparingly soluble ones, so long as they meet the objects of the invention, but there can, generally, be mentioned water-sparingly soluble or insoluble medicaments, or unstable medicaments.

Further suitable active ingredients are those which exert a local physiological effect, as well as those which exert a systemic effect, either after penetrating the mucosa or in the case of oral administration - after transport to the gastro-intestinal tract with saliva. The dosage forms prepared from the compositions according to the present invention are particularly suitable for active ingredients which exert their activ-

ity during an extended period of time, i.e. drugs having a half-life of at least several hours. Examples thereof are: analgesic and anti-inflammatory drugs (NSAIDs, flurbiprofen, fentanyl, indomethacin, ketoprofen, nabumetone, paracetamol, piroxicam, tramadol); anti-arrhythmic drugs (procainamide, quinidine, verapamil); antibacterial and antiprotozoal agents (amoxicillin, ampicillin, benzathine penicillin, benzylpenicillin, cefaclor, cefadroxil, cefprozil, cefuroxime axetil, cephalexin, chloramphenicol, chloroquine, ciprofloxacin, clarithromycin, clavulanic acid, clindamycin, doxycycline, erythromycin, flucloxacillin, halofantrine, isoniazid, kanamycin, lincomycin, mefloquine, minocycline, nafcillin, neomycin, norfloxacin, ofloxacin, oxacillin, phenoxymethyl-penicillin, pyrimethamine-sulfadoxime, streptomycin); anti-coagulants (warfarin); antidepressants (amitriptyline, amoxapine, butriptyline, clomipramine, desipramine, dothiepin, doxepin, fluoxetine, gepirone, imipramine, lithium carbonate, mianserin, milnacipran, nortriptyline, paroxetine, sertraline; 3-[2-[3,4-dihydrobenzofuro[3,2-c]pyridin-2(1H)-yl]-ethyl]-2-methyl-4H-pyrido[1,2-a]pyrimidin-4-one); anti-diabetic drugs (glibenclamide, metformin); anti-epileptic drugs (carbamazepine, clonazepam, ethosuximide, phenobarbitone, phenytoin, primidone, topiramate, valpromide); antifungal agents (amphotericin, clotrimazole, econazole, fluconazole, flucytosine, griseofulvin, itraconazole, ketoconazole, miconazole nitrate, nystatin, terbinafine, voriconazole); antihistamines (astemizole, cinnarizine, cyproheptadine, decarboethoxyloratadine, fexofenadine, lunarizine, levocabastine, loratadine, norastemizole, oxatomide, promethazine, terfenadine); anti-hypertensive drugs (captopril, enalapril, ketanserin, lisinopril, minoxidil, prazosin, ramipril, reserpine, terazosin); anti-muscarinic agents (atropine sulphate, hyoscine);

antivirals (acyclovir, AZT, ddC, ddI, ganciclovir, loviride, tivirapine, 3TC, delavirdine, indinavir, nelfinavir, ritonavir, saquinavir); antineoplastic agents and antimetabolites (adriamycine, cladribine, cisplatin, dactinomycin, daunorubicin, doxorubicin, etoposide, irinotecan, mitomycin, mitoxantrone, tamoxifen, taxol, taxotere, topotecan, trimetrexate, vincristine, vinblastine); anti-migraine drugs (almotriptan, alniditan, eletriptan, frovatriptan, naratriptan, rizatriptan, sumatriptan, zolmitriptan); anti-Parkinsonian drugs (bromocryptine mesylate, levodopa, selegiline); antipsychotic, hypnotic and sedating agents (alprazolam, buspirone, chlordiazepoxide, chlorpromazine, clozapine, diazepam, flupenthixol, fluphenazine, flurazepam, 9-hydroxyrisperidone, lorazepam, mazapertine, olanzapine, oxazepam, pimozide, pipamperone, piracetam, promazine, risperidone, selfotel, seroquel, sertindole, sulpiride, temazepam, thiothixene, triazolam, trifluperidol, ziprasidone, zolpidem); anti-stroke agents (lubeluzole, lubeluzole oxide, riluzole, aptiganel, eliprodil, remacemide); antitussive (dextromethorphan, laevodropropizine); beta-adrenoceptor blocking agents (atenolol, carvedilol, metoprolol, nebivolol, propanolol); cardiac inotropic agents (amrinone, digitoxin, digoxin, milrinone); corticosteroids (beclomethasone dipropionate, betamethasone, budesonide, dexamethasone, hydrocortisone, methylprednisolone, prednisolone, prednisone, triamcinolone); disinfectants (chlorhexidine); diuretics (acetazolamide, frusemide, hydrochlorothiazide, isosorbide); enzymes; essential oils (anethole, anise oil, caraway, cardamom, cassia oil, cineole, cinnamon oil, clove oil, coriander oil, dementholised mint oil, dill oil, eucalyptus oil, eugenol, ginger, lemon oil, mustard oil, neroli oil, nutmeg oil, orange oil, peppermint, sage, spearmint, terpineol, thyme); gastro-intestinal agents

(cimetidine, cisapride, clebopride, diphenoxylate, domperidone, famotidine, lansoprazole, loperamide, loperamide oxide, mesalazine, metoclopramide, mosapride, nizatidine, norcisapride, olsalazine, omeprazole, pantoprazole, perprazole, prucalopride, ranitidine, rabeprazole, ridogrel, sulphasalazine); haemostatics (aminocaproic acid); lipid regulating agents (atorvastatin, lovastatin, pravastatin, probucol, simvastatin); local anaesthetics (benzocaine, lignocaine); opioid analgesics (buprenorphine, codeine, dextromoramide, dihydrocodeine, hydrocodone, oxycodone, morphine); parasympathomimetics (eptastigmine, galanthamine, metrifonate, neostigmine, physostigmine, tacrine, donepezil, rivastigmine, milameline, sabcomeline, talsaclidine, xanomeline, memantine, lazabemide); sex hormones (oestrogens: conjugated oestrogens, ethinyloestradiol, mestranol, oestradiol, oestriol, oestrone; progestogens; chlormadinone acetate, cyproterone acetate, 17-deacetyl norgestimate, desogestrel, dienogest, dydrogesterone, ethynodiol diacetate, gestodene, 3-keto desogestrel, levonorgestrel, lynestrenol, medroxy-progesterone acetate, megestrol, norethindrone, norethindrone acetate, norethisterone, norethisterone acetate, norethynodrel, norgestimate, norgestrel, norgestrienone, progesterone, quingestanol acetate); stimulating agents (sildenafil); vasodilators (amlodipine, buflomedil, amyl nitrite, diltiazem, dipyridamole, glyceryl trinitrate, isosorbide dinitrate, lidoflazine, molsidomine, nicardipine, nifedipine, oxpentifylline, pentaerythritol tetranitrate).

In the preparation according to the invention, the compounding ratio between the acylated alkylated CyD and the medicament can be an any ratio so long as it meets the objects, but in view of controlling the release of the medicament from the preparation, the ratio of the acylated alkylated CyD : the medicament can be made to be 1 : 4 to 4 : 1, preferably 1 : 2 to 2 : 1, in

terms of mole ratio.

In the preparation of the invention, pharmaceutically acceptable other auxiliaries or additives can, if necessary, be incorporated in a range not to have bad influence on the objects of the invention. As such auxiliaries or additives, there can be mentioned stabilizers, dissolution aids, suspending agents, emulsifying agents, buffering agents, preservatives, isotonizing agents, or other proper additives, which are used regularly in the technical field.

The invention is specifically described below by examples, but the invention is not limited by these examples.

Example 1 Synthesis of DMA-β-CyD

DM-β-CyD (12 g) was dissolved in 60 mL of anhydrous pyridine, and 25 mg of 4-dimethylaminopyridine was added. Then, 12 mL of acetic anhydride was gradually added dropwise, and the mixture was subjected to reaction at 80℃ for 24 hours. After the completion of the reaction, the mixture was added dropwise into ice water to decompose the excess acid anhydride, and extracted with chloroform. Sodium carbonate was added to the organic phase to desalt it, and the mixture was subjected to separation and purification using a silica gel column. The obtained substance was concentrated to dryness to give the desired DMA-β-CyD (D.S. 7). This CyD derivative had a melting point of 113 to 117℃, and its solubility in water at 25℃ exceeded 50 mg/dl. The resulting DMA-β-CyD (D.S. 7) was recrystallized from water to give white crystals (yield 60%) having a melting point of 126℃. Its mass spectrum and ^{1}H-NMR spectrum are shown in Fig. 1 and Fig. 2, respectively.

FAB MS (negative mode) *m/z* 1777 [M+*m*-nitrobenzyl alcohol (matrix)-H); ^{1}H-NMR ($CDCl_3$) d 5.16 (t, 1H, CyD H-3), 5.00 (d, 1H, CyD H-1), 3.91-3.87 (m, 2H, CyD H-5 and H-6b), 3.79 (t, 1H, CyD H-4), 3.54 (d, 1H,

CyD H-6a), 3.37 (s, 3H, 6-CH_3), 3.33 (s, 3H, 2-CH_3), 3.21 (dd, 1H, CyD H-2), 2.04 (s, 3H, 3-CH_3).

Example 2 Synthesis of acetylated DM-β-CyD with a lower substitution at the 3-position

The acetylated DM-β-CyD was prepared by using a small amount of the acid anhydride (4.6 g, 45 mmol) to DM-β-CyD (10 g, 7.5 mmol). The other condition of preparation was identical to that described in Example 1, except for the recrystallization due to the face that it was a mixture of components with different D.S.s. The D.S. value was determined by a peak ratio of the CyD anomeric proton (H-1) and the methyl proton of acetyl groups in ^{1}H-NMR spectra (see Fig. 3), and was 3.8. The mixture is hereinafter referred to as DMA4-β-CyD.

Example 3 Synthesis of butyrated DM-β-CyD

DM-β-CyD (5 g) was dissolved in 25 mL of anhydrous pyridine, and 9 mL of n-butyric anhydride was added, and the mixture was subjected to reaction at 80℃ for 24 hours. After the completion of the reaction, the mixture was added dropwise into ice water to decompose the excess acid anhydride, and extracted with chloroform. Sodium carbonate was added to the extract to desalt it, and the mixture was subjected to separation and purification using a silica gel column. The obtained substance was concentrated to dryness to give the desired butyrated DM-β-CyD. This CyD derivative had a melting point of 108 to 111℃. Its ^{1}H-NMR spectrum is shown in Fig.4.

Example 4 Synthesis of octanoylated DM-β-CyD

DM-β-CyD (5 g) was dissolved in 25 mL of anhydrous pyridine, and 16 mL of octanoic anhydride was added, and the mixture was subjected to reaction at 80℃ for 24 hours. After the completion of the reaction, the mixture was added dropwise into ice water to decompose the excess acid anhydride, and extracted with chloroform. Sodium carbonate was added to the extract to

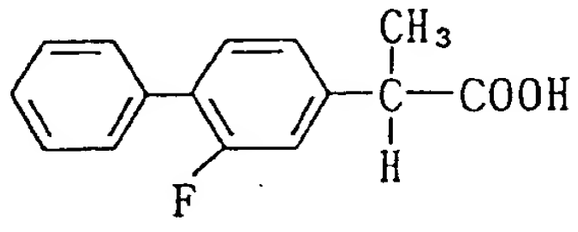
desalt it, and the mixture was subjected to separation and purification using a silica gel column. The obtained substance was concentrated to dryness to give the desired octanoylated DM-β-CyD. This CyD derivative was an oily substance at 25℃. Its ^{1}H-NMR spectrum is shown in Fig. 5.

Characteristic tests

(1) Determination of stability constant of DMA-β-CyD

In this test, the inclusion properties of DMA-β-CyD was compared with those of the parent β-CyD and DM- and TM-β-CyDs.

The stability constant between DMA-β-CyD and flurbiprofen was determined by the solubility method i.e., according to the method of Higuchi, T. et al., Adv. Anal. Chem. Instr. 1965, 4, 117-212. The stability constants between β-CyD, DM-β-CyD or TM-β-CyD and flurbiprofen determined simultaneously for comparison are also shown in Table 1.



Flurbiprofen

Table 1:

Compound	Stability constant (M^{-1})
β-CyD	3613
DM-β-CyD	8055
TM-β-CyD	1655
DMA-β-CyD	1212

These results suggest that DMA-β-CyD has the same inclusion ability as TM-β-CyD, although it is inferior in inclusion ability to DM-β-CyD.

(2) Test on hemolytic properties of DMA-β-CyD and DMA4-β-CyD

In this test on hemolytic properties, 6 to 7 mL of blood was taken from the auricular vein of a white rabbit, 1 mL of a preserved erythrocyte solution was added, and the mixture was gently mixed. To this was added 4 to 5 mL of 10 mM isotonized phosphate buffer (pH 7.4), the mixture was gently mixed and centrifuged at 1,000 g for 5 minutes, and the supernatant was removed. This washing operation was repeated three times, 10 mM isotonized phosphate buffer (pH 7.4) was added to 1 mL of the resultant solution to make the volume 20 mL, and thereby a 5 % erythrocyte suspension was prepared.

The CyD derivative of various concentrations was diluted with 10 mM isotonized phosphate buffer (pH 7.4), and the resultant each dilution was incubated at 37℃. Then 4 mL of this dilution was taken, 0.2 mL of the 5 % erythrocyte suspension was added, and the mixture was incubated at 37℃ for 30 minutes. The mixture was centrifuged at 1,000 g for 5 minutes, 3 mL of the supernatant was measured for absorbance at 543 nm, and thereby its hemolytic activity was determined. Further, the specimen was observed visually using a microscope. The obtained results are shown in Fig. 6. To the 5 % erythrocyte suspension was added 100 mM DMA-β-CyD (D.S. 7), DMA4-β-CyD (D.S. 3.8), β-CyD, DM-β-CyD, TM-β-CyD, 2-HP-β-CyD (D.S. 4.8) (see Shiotani, K. et al. Pharm. Res. 1995, 12, 78-84) or subfobutyl ether β-CyD (D.S. 3.5)

(see ibid), the above treatment was carried out, and then observation by a microscope was made.

It is apparent that the hemolytic activity of DMA-β-CyDs was weaker than those of β-CyD, DM-β-CyD and TM-β-CyD. For example, the hemolysis began at about 2 mM, 0.5 mM and 1 mM, and the concentrations to induce 50% hemolysis were about 4 mM, 1 mM and 2 mM for β-CyD, DM-β-CyD and TM-β-CyD, respectively. On the other hand, the hemolysis of DMA4-β-CyD with D.S. 3.8 began at about 12 mM, and its 50% hemolysis concentration was about 22 mM. In the case of DMA-β-CyD with D.S. 7, no hemolyis was observed up to 100 mM. The hemolytic activity of DMA-β-CyDs was weaker than those of 2-HP (D.S. 4.8) and sulfobutyl ether of β-CyD (D.S. 3.5).

(3) Determination of the released amount of cholesterol from the intact erythrocytes of rabbits treated with DMA-β-CyD or DMA4-β-CyD

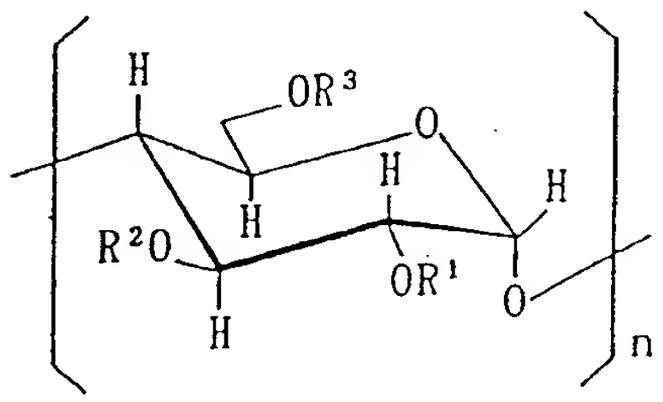
Each DMA-β-CyD, DMA4-β-CyD, DM-β-CyD was diluted with 10 mM isotonized phosphate buffer (pH 7.4), and the dilution was incubated at 37℃. Then, 4 mL of the dilution was taken, 0.2 mL of the 5 % erythrocyte suspension was added, and the mixture was incubated at 37℃ for 30 minutes. The mixture was centrifuged at 1,000 g for 5 minutes, 5 mL of chloroform was added to 3 mL of the supernatant, and the mixture was shaken for 30 minutes to make extraction. The chloroform layer was taken, and concentrated to give a specimen. The specimen was assayed for cholesterol amount using a Cholesterol E-Test Wako (made by Wako Pure Chemical Industries, Ltd.). The obtained results are shown in Fig. 7.

One of the causes of CyD-induced hemolysis is

known to be extractions of cholesterol and phospholipids from erythrocytes through the inclusion complex formation. Therefore, in this test, the cholesterol release behavior from rabbit erythrocytes by the addition of DMA-β-CyD or DMA4-β-CyD was investigated and compared with the cholesterol release behaviors of a control (an isotonic buffer without CyDs) and DM-β-CyD. Figure 7 shows the released amounts of cholesterol from the intact erythrocytes of rabbits treated with β-CyDs in 10 mM phosphate buffer (pH 7.4) at 37℃. DM-β-CyD induced about 80% release of cholesterol at a concentration of 0.5 mM at which the hemolyis only slightly occurred (see Figure 6). On the other hand, DMA-β-CyDs induced only 10% release of cholesterol at the same concentration, and this release was the same as that of the control experiment conducted in the isotonic buffer.

CLAIMS

1. An acylated alkylated cyclodextrin derivative represented by the formula (I)



(I)

wherein n is any of integers 6, 7 and 8, and the 1-position and the 4-position of the sugar residues at the both ends are mutually bound by a covalent bond,

R^1, R^2 and R^3 independently represent hydrogen atoms, lower alkyl groups or C_{2-20} alkanoyl groups, or in some case, represent glucosyl groups or maltosyl groups whose hydroxyl group(s) may be replaced with lower alkyloxy group(s) or C_{2-20} alkanyloxy group(s),

provided that any of R^1, R^2 and R^3 of the number of total $3 \times n$ composed of each n are simultaneously at least one lower alkyl group and at least one C_{2-20} alkanoyl group, and the residual groups, when exist, are hydrogen atoms or the glucosyl groups or maltosyl groups of the number of up to at most 2,

or a mixture of two or more of the derivatives.

2. The acylated alkylated cyclodextrin derivative

or mixture of two or more of the derivatives according to claim 1 wherein 50 % or more of R^1 and R^3 of the number of total 2×n are lower alkyl groups, the residual R^1 and R^3 and R^2 are at least one C_{2-20} alkanoyl group, and the residual R^1, R^2 and R^3, when exist, are hydrogen atoms.

3. The acylated alkylated cyclodextrin derivative or mixture of two or more of the derivatives according to claim 1 wherein R^1 and R^3 are lower alkyl groups, 50 % or more of R^2 are C_{2-20} alkanoyl groups, and the residual R^2, when exist, are hydrogen atoms.

4. The acylated alkylated cyclodextrin derivative or mixture of two or more of the derivatives according to claim 1 wherein R^1 and R^3 are lower alkyl groups, and 50 % or more of R^2 of the number of n are acyl groups.

5. The acylated alkylated cyclodextrin derivative or mixture of two or more of the derivatives according to any of claims 1 to 4 wherein the lower alkyl groups are methyl, ethyl, n-propyl, isopropyl, n-butyl or sec-butyl, and the alkanoyl groups are acetyl, n-propanoyl, n-butanoyl, n-pentanoyl, n-hexanoyl, n-heptanoyl, n-octanoyl, n-dodecanoyl, n-tetradecanoyl or n-octadecanoyl.

6. The acylated alkylated cyclodextrin derivative or mixture of two or more of the derivatives according to any of claims 1 to 5 wherein the lower alkyl groups are methyl groups, and the alkanoyl groups are acetyl groups.

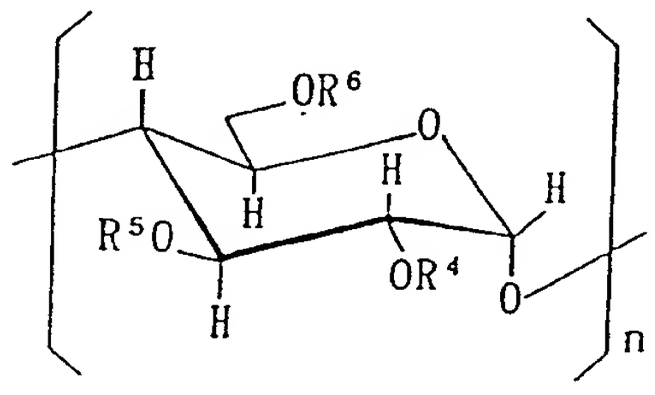
7. The acylated alkylated cyclodextrin derivative or mixture of two or more of the derivatives according to any of claims 1 to 6 wherein n is 7 in the formula

(I).

8. Heptakis (2,6-di-O-methyl-3-O-acetyl)-β-cyclodextrine; heptakis (2,6-di-O-methyl-3-O-butyryl)-β-cyclodextrine; or heptakis (2,6-di-O-methyl-3-O-octanoyl)-β-cyclodextrine.

9. A mixture of acetylated heptakis (2,6-di-O-methyl)-β-cyclodextrines having an average degree of acetyl-substitution of about 3.8 at the 3-position.

10. A process for preparing an acylated alkylated cyclodextrin derivative represented by the formula (I) according to claim 1 or a mixture of two or more of the derivatives which comprises reacting a partially alkylated cyclodextrin derivative represented by the formula (II)



(II)

wherein n is any of integers 6, 7 and 8, and the 1-position and the 4-position of the sugar residues at the both ends are mutually bound by a covalent bond,

R^4, R^5 and R^6 independently represent hydrogen atoms, lower alkyl groups, glucosyl groups or maltosyl groups, provided that R^4, R^5 and R^6 of the number of total $3 \times n$ composed of each n are, simultaneously, at least one lower alkyl group and at least one hydrogen atom, and the number of the glucosyl groups

and maltosyl groups is at most 2,

or a mixture of the derivatives with an activated C_{2-20} alkanoic acid in a polar solvent, if necessary in the presence of a condensing agent to acylate the compound(s) of the formula (II).

11. A pharmaceutical preparation which comprises the acylated alkylated cyclodextrin derivative or mixture of two or more of the derivatives according to any of claims 1 to 9 and a medicament in such a state that they are closely compounded.

12. The pharmaceutical preparation according to claim 11 wherein the medicament is selected from the group consisting of nonsteroidal antirheumatic agents, steroids, cardiac glycosides, benzodiazepine derivatives, benzimidazole derivatives, piperidine derivatives, piperazine derivatives, imidazole derivatives and triazole derivatives.

13. A process for preparing a pharmaceutical preparation according to claim 11 or 12 which comprises kneading closely the acylated alkylated cyclodextrin derivative or mixture of two or more of the derivatives according to any of claims 1 to 6 and a medicament in an aqueous solvent, and then, if necessary, removing the solvent.

FIG. 1

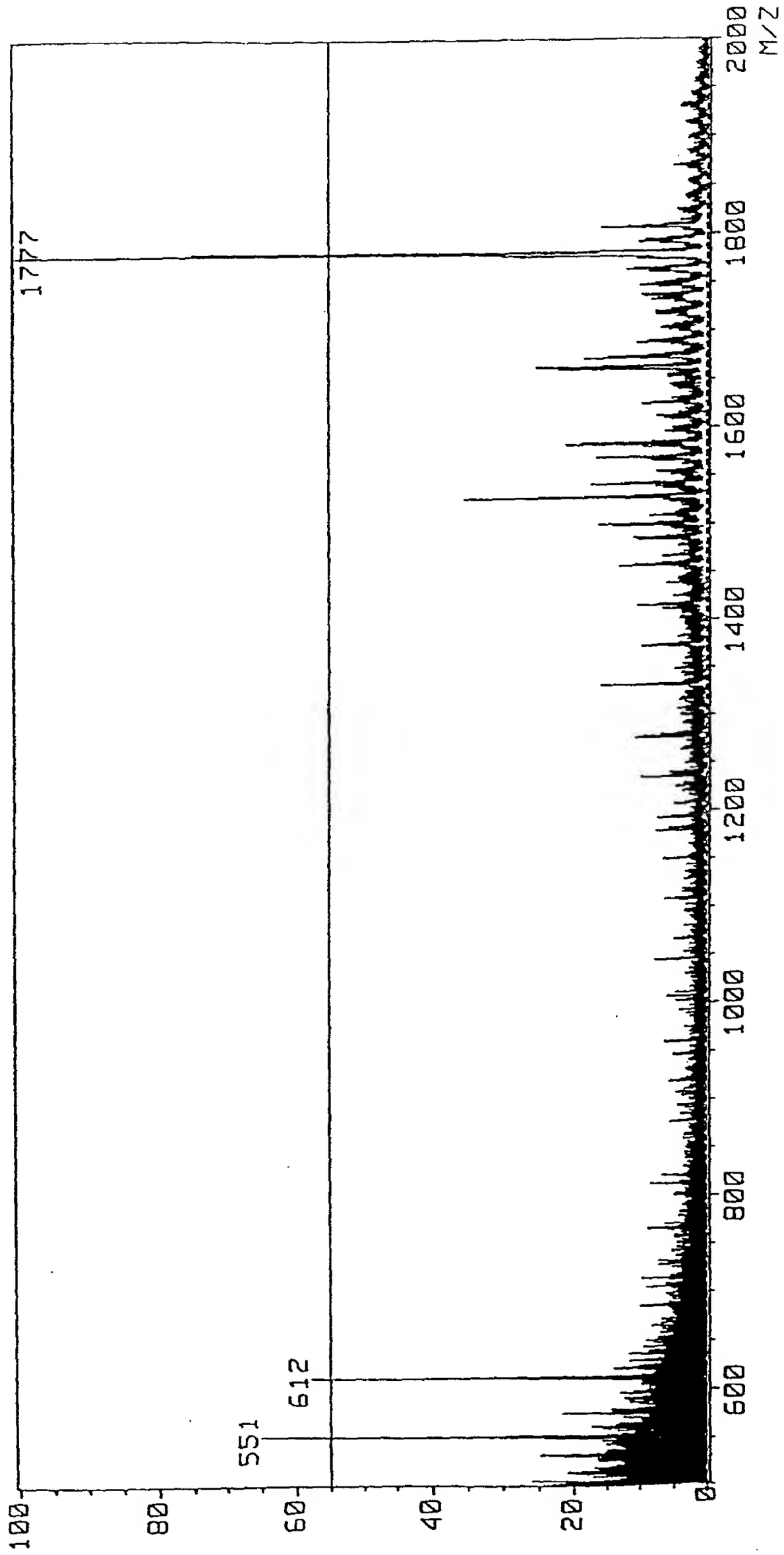

2000
M/Z
1800
1600
1400
1200
1000
800
600
1747
612
551
100
80
60
40
20
0

FIG. 2

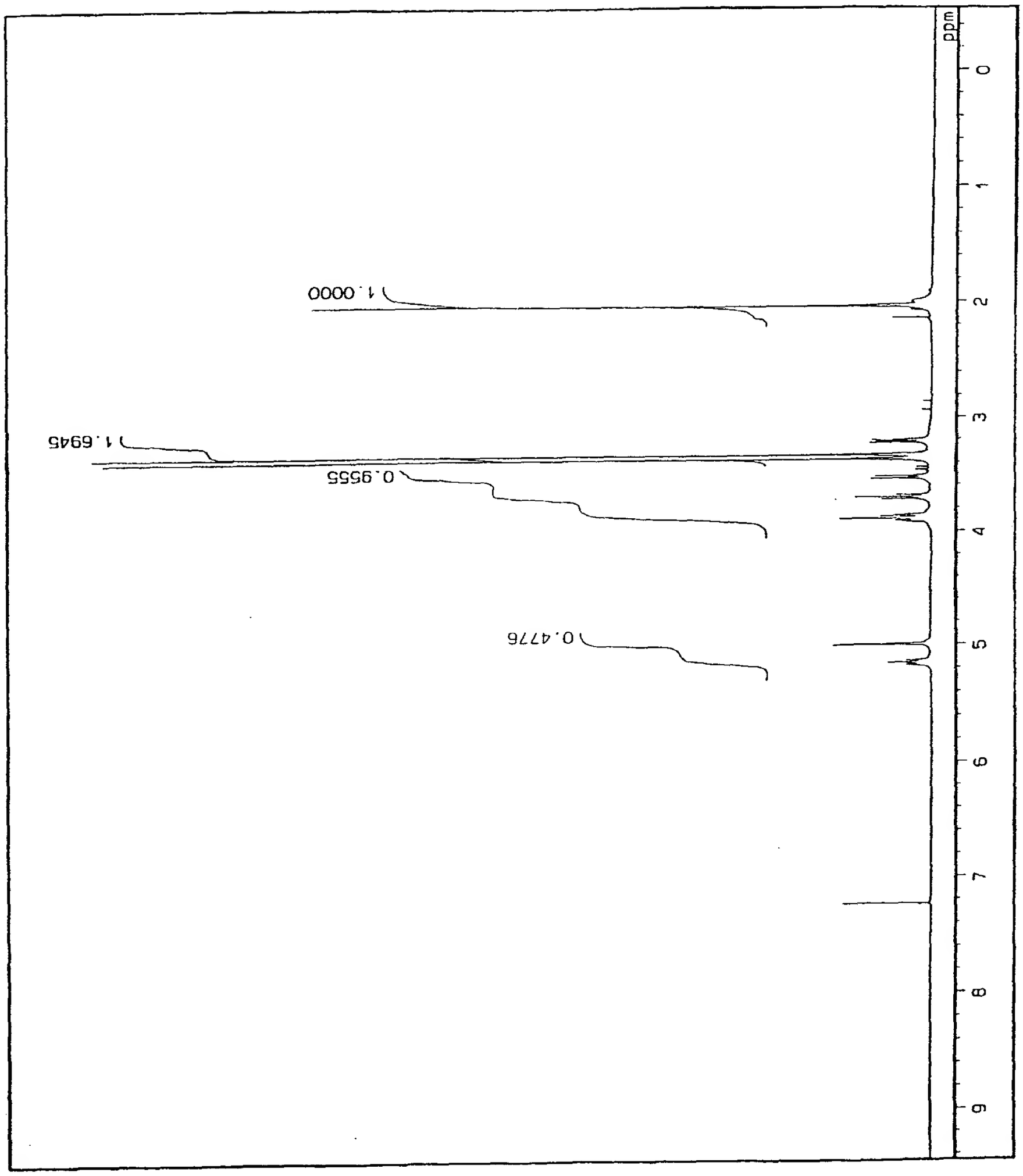


FIG. 3

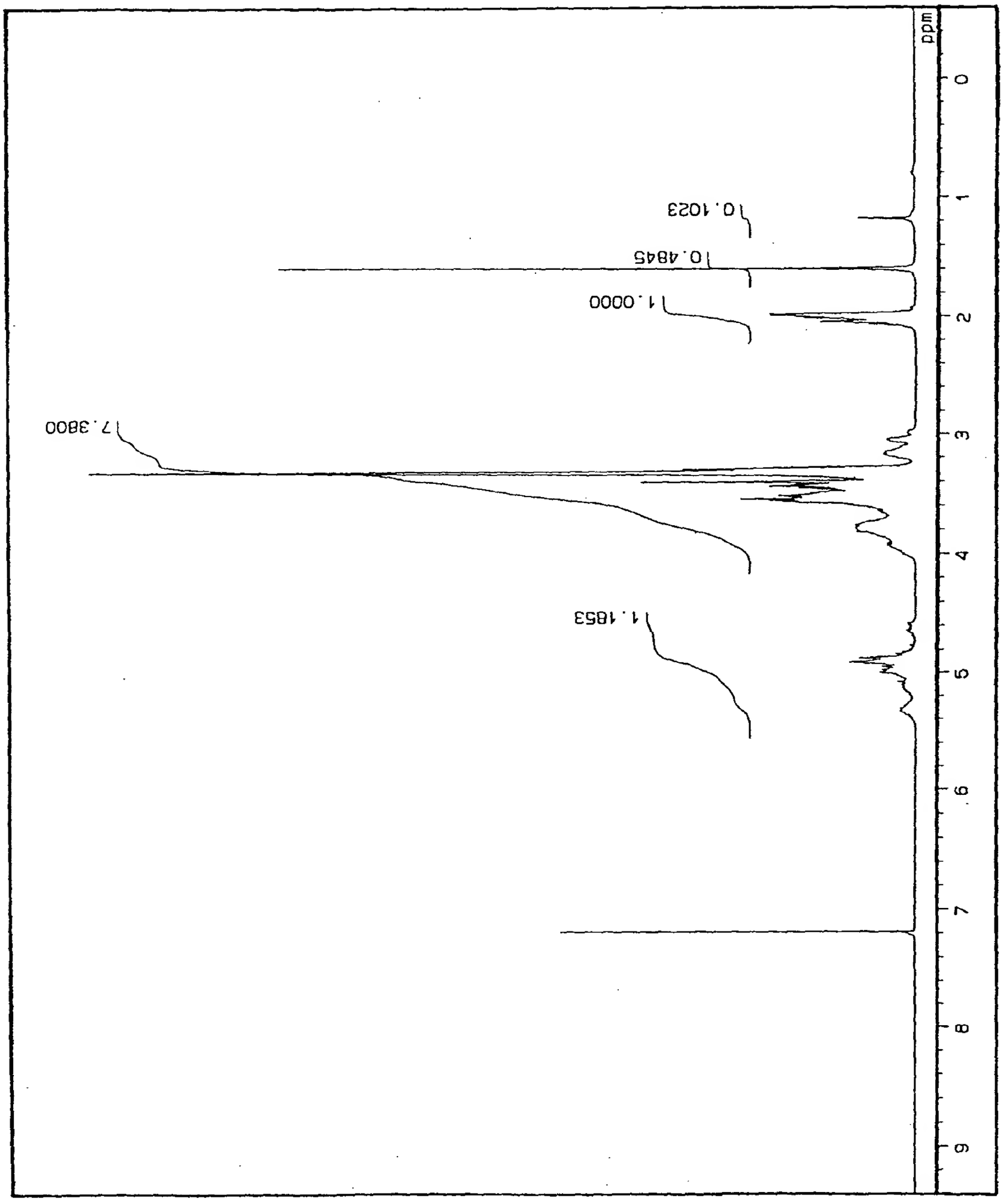


FIG. 4

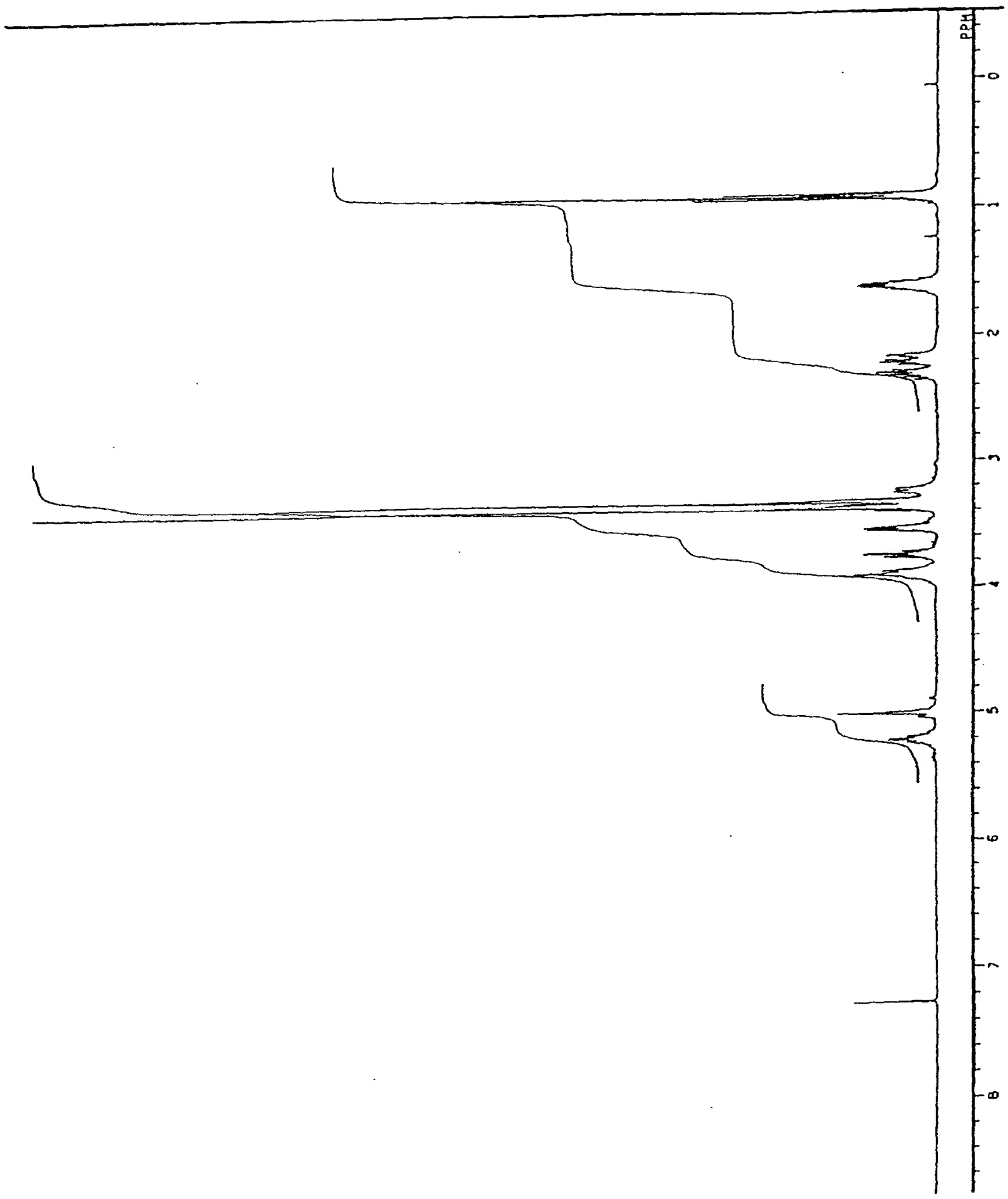


FIG. 5

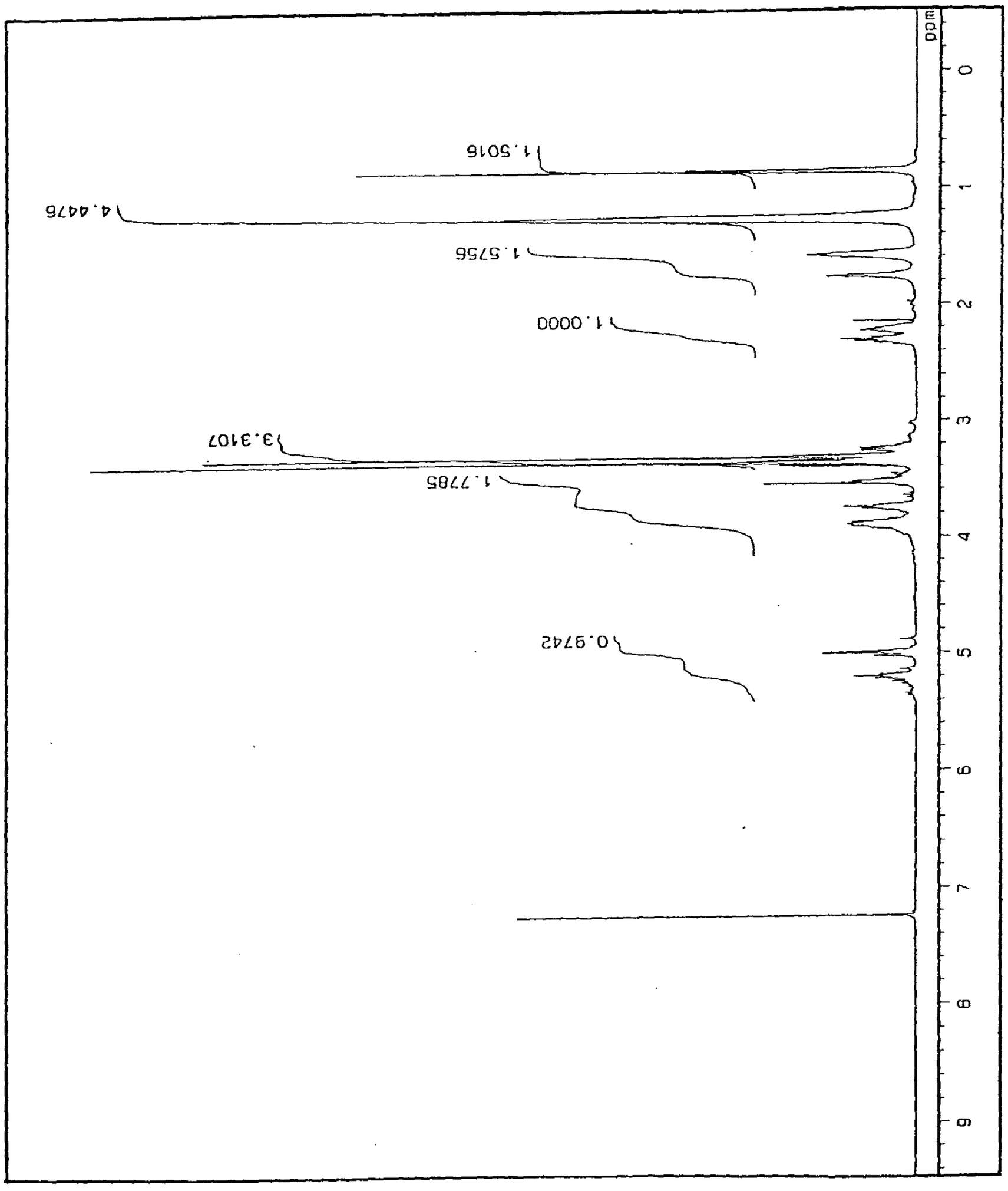


FIG. 6

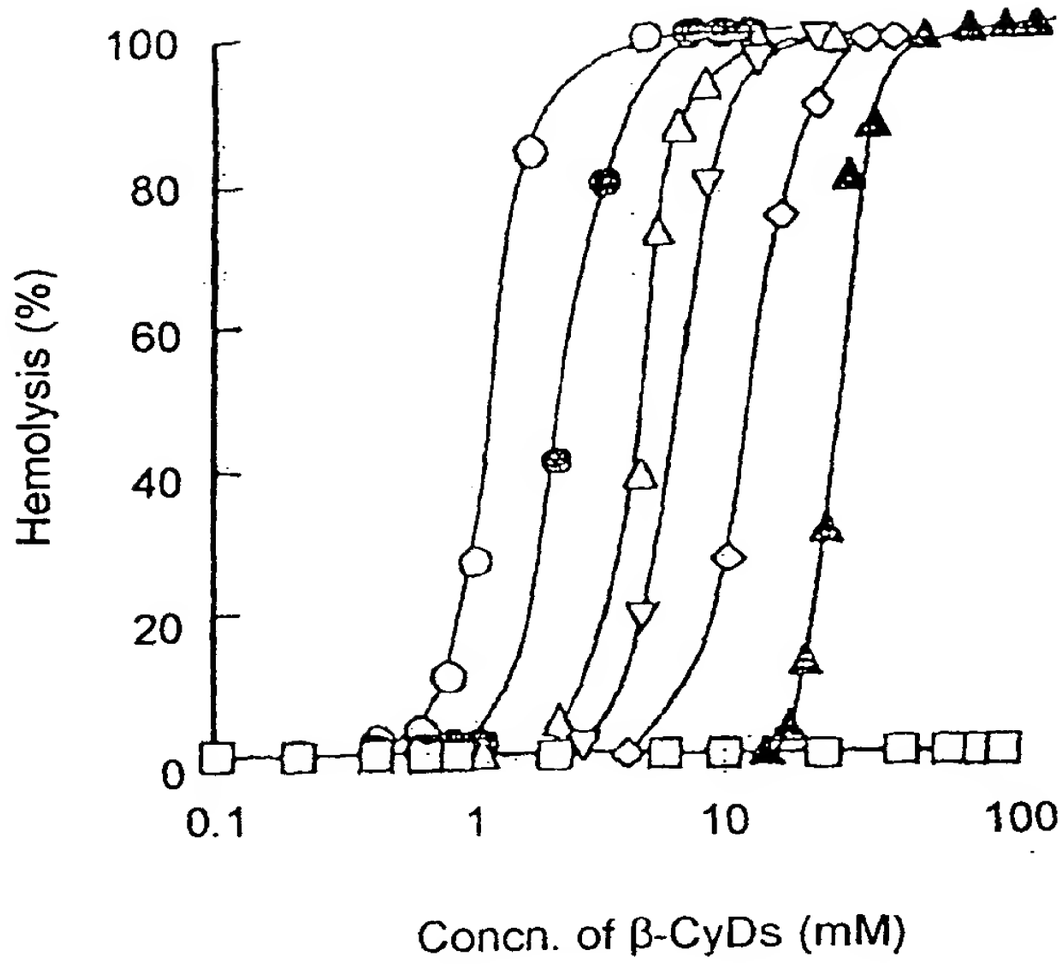
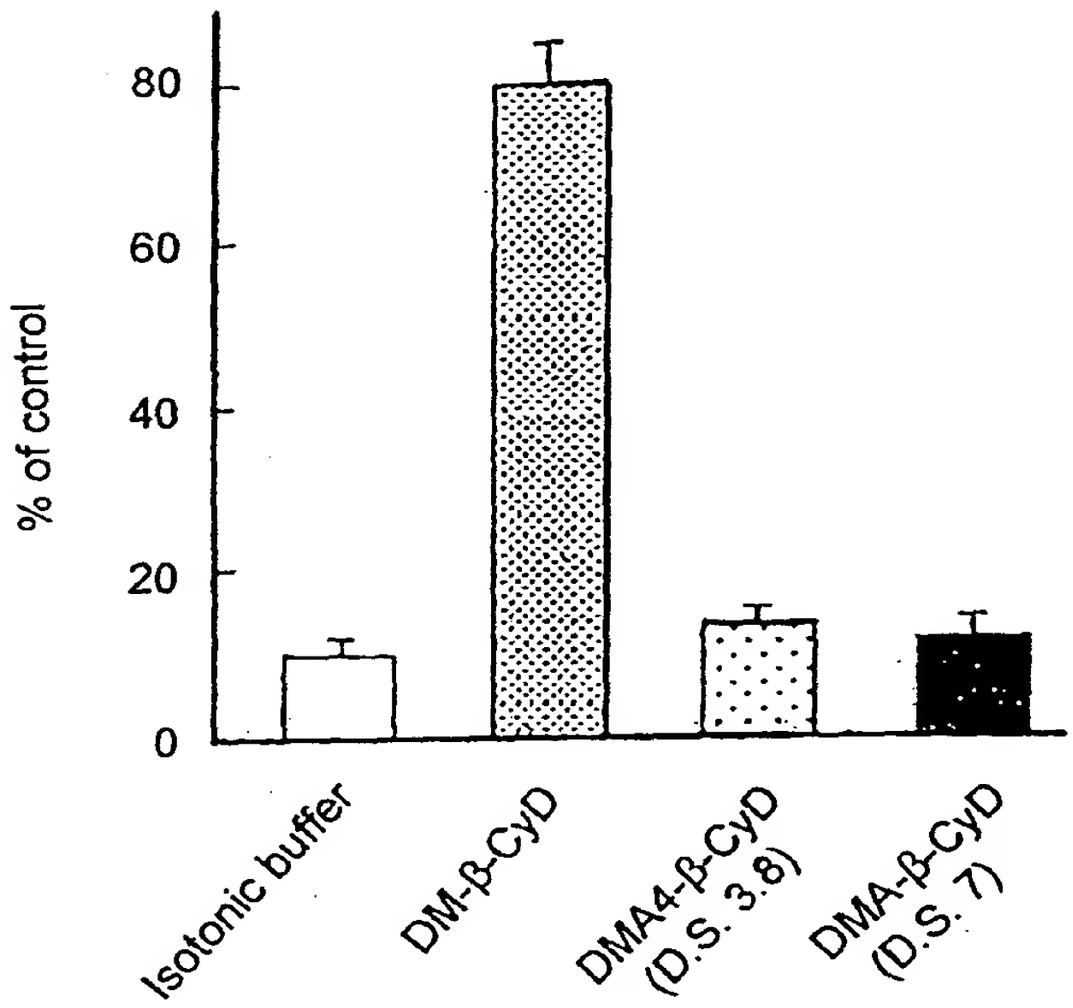

100
80
60
40
20
0
Hemolysis (%)
0.1
1
10
100
Concn. of β-CyDs (mM)

FIG. 7


80
60
40
20
0
% of control
Isotonic buffer
DM-β-CyD
DMA4-β-CyD (D.S. 3.8)
DMA-β-CyD (D.S. 7)

INTERNATIONAL SEARCH REPORT

Inter: ··, ,nal Application No
PCT/JP 99/02806

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C08B37/16 A61K47/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C08B A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 89 09235 A (MACHEREY-NAGEL GMBH) 5 October 1989 (1989-10-05) claims 1-7; example 3	1-4,10
X	EP 0 312 352 A (CHINOIN) 19 April 1989 (1989-04-19) page 3, line 17,18 page 13, line 60 - line 64 page 14, line 34 - line 40	1-13

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 August 1999	18/08/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Lensen, H

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INTERNATIONAL SEARCH REPORT

information on patent family members

Intern ational Application No
PCT/JP 99/02806

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 8909235 A	05-10-1989	DE 3810737 A	12-10-1989
		EP 0407412 A	16-01-1991
		JP 3505337 T	21-11-1991
		US RE36092 E	09-02-1999
		US 5198429 A	30-03-1993
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		US 5008386 A	16-04-1991